THE COLORING MATTER OF THE ARIL OF CELASTRUS SCANDENS.

BY IDA A. KELLER.

The presence of different pigments manufactured by the vegetable organism has forced the plant world upon the attention of the human race from time immemorial. If we submit the colored parts to microscopical examination we are usually confronted by one of two distinct cases.

Firstly, we may find that the pigment, instead of pervading the entire cell, is found only in certain variously shaped bodies which are more or less regularly scattered through the cell contents. The best known illustration of this kind is to be found in ordinary leaves, the green color being confined to the chlorophyll granule. Secondly, if we examine other parts of plants we may find that the coloring matter is distributed uniformly throughout the cell sap. The blue flower of the Grape Hyacinth may serve as one of the many illustrations of the latter case. Wherever fixed and definite portions of protoplasm subserve a special function within the plant cell, these may be considered as parts of a unit and they may be termed organs of the cell. In addition, then, to the nucleus we may find various other organs as, for example, the colored bodies just referred to. A distinction must be made between such differentiated portions of the protoplasm and the products which are the result of their activity, between the colorless protoplasmic matrix and the colored product which makes it conspicuous. If we observe e. g., a living cell of a leaf of *Elodea Canadensis* we find as organs of the protoplasmic contents the nucleus and the chlorophyll granules ; as a product of the latter, chlorophyll and finally starch as a result of the action of the chlorophyll in response to satisfactory external conditions. Such conditions are a certain amount of heat, light, moisture and the absence of any injurious factors which might impede the various operations manifested in life activity.

In dealing with the products of this activity we come to a problem of great complexity. It is true that certain phenomena as witnessed in the vegetable cell can be explained by known principles of physics and chemistry, and that many substances for which mankind was formerly dependent on the vegetable organism are now manufactured in the chemical laboratory. I need only recall the

synthetic preparation of alizarin, alcohol, indigo, oxalic, citric, tartaric and salicylic acids, vanillin and finally sugars, to call to mind a host of further illustrations. On the other hand it must be admitted that this victory, great as it is, has sometimes been overrated and has tended to make the scientist overbearing as shown by his attempts to resolve the phenomena of life into a simple operation of chemical and physical forces, without taking duly into consideration the highly organized structure of the protoplasmic mass, whose harmonious operation with a set of external conditions is manifested by what we call life. It is because of the exceedingly intricate mechanism of the protoplasmic structure, of whose operations we know very little, that our knowledge of the products of its activity is still extremely incomplete. Only in such cases, when we can obtain products capable of crystallizing, can we with any certainty state that we have to deal with chemical individuals whose formulas may be ascertained. If amorphous we cannot be sure but that we have instead of one, a mixture of substances more or less closely allied.

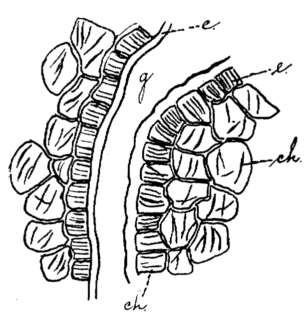
Before going further in the discussion of these plant products a few more words should be said in regard to the organs which bear the colors. The protoplasmic corpuscles have been appropriately designated chromatophores, which name is now generally accepted. It has been observed that as a rule, yellow, orange and brown (sometimes blue) coloring matters are deposited in such chromatophores, while white, violet, blue and red (sometimes yellow) are usually caused by a solution of the pigment in the cell sap. It has been found desirable to make a distinction between the kinds of chromatophores. They are for convenience classified as follows: chloroplasts, chromoplasts and leucoplasts, the latter class, which are the colorless color bearers, being one of the contradictions in which the systems of human classification abound. The bond of sympathy is, however, their common origin, the fact that one may be converted into the other according to the conditions, and each one can originate only as a result of the division of pre-existing chromatophores.

Chloroplasts, as their name indicates, are the green bodies which impart the green color characteristic of leaves and stems. The pigment in this case can be readily extracted by means of such solvents as alcohol, ether and chloroform, while the matrix remains behind as a definitely shaped, colorless mass of protoplasm. The pigment itself may under the influence of various factors, external or internal, undergo modifications into chemically different substances, such as etiolin.

Chromoplasts include all colored chromatophores, not green. It may be seen from this that the distinction is quite an arbitrary one. Chromoplasts may originate from leucoplasts or chloroplasts. This latter case can be easily observed in the ripening of many fruits, as they change from green to red, for example, apples or the berries of the potato plant.

As indicated by the variety of colors found in plants we have to deal with a number of chemically different substances. The literature existing on these pigments is not very satisfactory. Although the metamorphosis of the chloroplasts into the chromoplasts may be readily observed the new substances resulting from this metamorphosis are not well known. This past summer I became somewhat interested in the red color of fruits and collecting among others those of *Ilex verticillata*, I found that they turn brown in 50 per cent alcohol, those of *Gaultheria procumbens* turn gray; those of *Magnolia glauca*, dark brown; those of *Lindera Benzoin*, almost black; those of *Berberis Thunbergii*, light brown; those of *Cratægus coccinea*, dark brown. It is a matter of general observation that in most cases when immersed in alcohol the red color disappears and changes to gray, black or intermediate tints and this no doubt is due to a process of oxidation of the pigment. In rare instances, however, the red color does not seem to be affected by alcohol as, for examples, the berry of *Arisœma triphyllum* and the aril of the seed of *Celastrus scandens*. The latter I determined to submit to microscopical and chemical examination and the following are the results of my observations.

The coloring matter in this case occurs in chromatophores. The figure reveals the following anatomical structure:—A very much



thickened cuticle (*c*) of a lemon yellow color. This without a doubt affects to some extent the tint of the aril which has some yellow in it. Courchet[1] states that the color of certain fruits is entirely due to the impregnation of pigment in such epidermal thickenings and he cites as illustrations *Solanum macrocarpum* and *S. racemiflorum*. The epidermis (*e*) consists of a layer of smaller cells of a rather uniform size. The chromato-

[1] Courchet. Recherches sur les chromoleucites, Annales de Sc. Nat., Bot. VII, Ser. VII, 1888, p. 301.

phores (*ch*) within these are very conspicuous. They are rather closely packed together and lie parallel to each other. In color they are bright red, and in form very narrowly spindle-shaped. Below the epidermis, the cells constituting the rest of the pulp of the aril are of larger dimensions, and the chromatophores seem scattered irregularly through the cells. The drawing shows also the groove (*g*) between the arils of two adjoining seeds. Attention has been called to the fact that the study of chromatophores and pigments can be carried on with entire certainty only within the living cells on account of their ready decomposition. When I collected my material I had not the opportunity of careful examination, but the resistance which this tissue manifests to powerful reagents, leads me to conclude that in all probability the arrangement as above described is identical with that of the living material. I found further that sections from the dried seeds did not show any difference in appearance from that represented in the drawing.

According to Zimmermann[2] the pigments of chromatophores found in phanerogams, regarding which we have somewhat definite descriptions, are as follows:

1. Chlorophyll green.
2. Carotin including chlorophyll yellow.
3. Xanthin.
4. Coloring matter of Aloe flowers.

Although certain reactions are characteristic of each of these four pigments, and although an abundant literature exists, at least so far as the first of these, chlorophyll green, is concerned, we can not with any justification claim even such knowledge as the chemist has in reference to many organic compounds of the various complex series. A formula is attempted only for carotin which is said to be $C_{26} H_{38}$. The great difficulty in investigating these pigments lies in their unwillingness to crystallize. Carotin is the only one of these four which occurs within the vegetable cell in crystalline form, and which can be again crystallized when extracted from the plant. In regard to amorphous extractions complete certainty is always wanting as to the purity of the product, i. e., whether we have a chemical individual to deal with or with a mixture of more or less closely related compounds.

[2] Zimmermann, Botanical Microtechnique. Translated by James Ellis Humphrey, N. Y., 1893.

In spite of these discouraging facts this field of research seems to me well worth especial labor and care and the only feasible method is to continue the careful investigations of Arnaud, Courchet, Immendorff and Zimmermann which will no doubt shed further light on this hitherto dark field, of interest alike to the botanist, chemist and physiologist.

I selected the aril of the seed of *Celastrus scandens*, since some of the peculiarities of the pigment are well marked and I desired to find if possible its place in Zimmermann's four pigments.

Carotin is found as a crystalline secretion in the root of *Daucus Carota* also in red flowers and fruits of other plants. It imparts a blood red color to carbon bisulphide in which it is readily soluble and from which it may be obtained in the form of a crystalline precipitate by the addition of alcohol. I found that the pigment of the aril of *Celastrus scandens* was soluble in carbon bisulphide forming a deep red solution, but no precipitate was visible in the addition of alcohol. After evaporation an amorphous sticky mass resulted and it will thus be seen that it differs from carotin in this respect.

In using various well known solvents I found their effects as follows:

1. Water, no visible effect.
2. Alcohol, 50 per cent no visible effect on chromatophores, but the solution was slightly tinged yellow.
3. Alcohol absolute, more soluble; the solution of a deeper tinge.
4. Ether, about like 50 per cent alcohol in color but a greater amount of yellow residue left on evaporation.[3]
5. Aceton, about like 50 per cent alcohol.
6. Chloroform, much more soluble, solution deep red.
7. Carbon bisulphide, similar to chloroform, solution deep red.

Carotin "according to Arnaud is insoluble in water, almost so in alcohol, very slightly soluble in ether, and most so in chloroform and carbon bisulphide. These solutions are colored yellow to orange yellow, according to their degree of concentration, while the solution of carotin in carbon bisulphide is always blood red." [4]

---

[3] It is possible that the yellow matter with which the cuticle is impregnated influences to some extent the color imparted to the solvents. This requires further attention.

[4] Zimmermann, Microtechnique, p. 102.

Comparing then this statement with what I have observed regarding the pigment under consideration we find that there is a close similarity as to its solubility and that of carotin.

With concentrated sulphuric acid the chromatophores changed first to a greenish color and then to a decidedly purple-blue. This same change of color was effected when concentrated sulphuric acid was added to the chloroform solution. With iodine (in potassium iodide) the chromatophores turned blue-green, like the color characteristic of the Cyanophyceæ.

According to Zimmermann[5] with a solution of iodine (e. g. aqueous solution of iodine and iodide of potassium) carotin is colored greenish or greenish-yellow; with concentrated sulphuric acid, first violet and then indigo blue.

There is evidently, therefore, also much resemblance between the effect of iodine and concentrated sulphuric acid upon carotin and the red pigment of *Celastrus scandens.*

Lacking, however, complete correspondence I next determined to discover if it approached xanthin more closely in its properties. It differs from this in its most conspicuous, although on that account by no means most important property, its color. "Xanthin occurs in yellow chromoplasts in amorphous form, and especially in small granules.[6] Its alcoholic solution leaves on evaporation a wholly amorphous resin-like mass. It is insoluble in water, little soluble in ether, chloroform and benzine but more so in alcohol. With concentrated sulphuric acid, the isolated pigment, as well as the chromoplast takes first a greenish then a blue color; with iodine best used in the form of potassium iodide it becomes green."[7]

It will be seen from this that while the red pigment of *Celastrus scandens* differs from xanthin in its solubility it agrees with it more closely as regards the effect of sulphuric acid than does carotin. Another striking resemblance with xanthin is the resin-like amorphous residue left when the solvents are evaporated.

The behavior of the coloring matter of the aril of the seed of *Celastrus scandens* with different solvents and other reagents leads

[5] Ibid., p. 102.

[6] It appears to me of no great importance to distinguish between pigments occurring in solution or in granules so long as we know no more about solutions than we do at present. We consider pigments in solution if present in such a fine state of division that the individual particles can no longer be recognized. It must be admitted than such an distinction is purely arbitrary.

[7] Zimmermann, Microtechnique, p. 103.

us to conclude that in it we find a connecting link between the crystallizing carotin of red flowers and fruits and the amorphous resin-like xanthin of yellow flowers, and these observations tend to confirm Courchet's views that the pigments of yellow and red chromatophores having the property of turning blue or green with sulphuric acid, thus distinguished from all other pigments, represent a group of closely related compounds[8] whose composition certainly demands further investigation.[9]

---

[8] Courchet, Recherches sur les chromoleucites. Annales de Sc. Nat., Bot. VII Ser. VII, 1888, p. 291.

[9] The coloring matter described in this paper is also remarkable for its resistance to the action of alkalies. Boiling with potassium hydroxide does not decompose it.